NIEHS Users Group Meeting

Sample Submission and Recent Advances in the Microarray Center

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Outline for Today's Talk

- Website Updates
- RNA Isolation and Submission
- RNA Labeling Methods
- High Throughput Sequencing
- Scanning and Image Analysis Improvements
- Printing Modifications
- The New Human Oligo Chip

Website Updates

- New Look
- More Protocols
- PDF Format
- FAQs
- Search Features

Dir.niehs.nih.gov/microarray

RNA Isolation and Submission

- Tissue Culture
- Liver, Kidney, Thymus, Lung, Prostate, and Ovary
- Skin
- Poly A selection coming soon

RNA Isolation and Submission

Sample Submission Requirements
(samples will be returned if all criteria are not met):

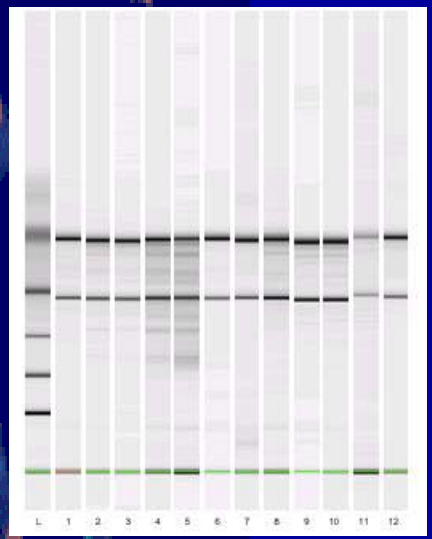
Please check boxes when criteria are met.

- ☐ **a**. RNA concentration of a minimum: 5 ug/ul for total RNA or 0.5 ug/ul for Poly A +RNA, in nuclease-free water.
- ☐ **b**. Minimum submission of 200 ug total RNA or 15 ug Poly A + RNA per comparision (i.e. if a sample, such as a control, is to be compared to more than one other sample, there must be 200 ug total RNA per comparison). Reserve sufficient quantity for signal validation by traditional methods, e.g. northern blot.
- ☐ **c**. OD 260/280 absorbance ratio of 1.7-2.1, quantitated **using NMC RNA quantitation protocol.** Please see website for current protocols: http://dir.niehs.nih.gov/microarray/
- ☐ **d**. A 5 uL sample at 100ng/ul of each RNA sample submitted for RNA quality check on Agilent Bioanalyzer.
- ☐ **e**. Submission must be accompanied by **original** gel image (clearly labeled and easily distinguishable) with 1-5 ug/lane of each total RNA species. If submitting mRNA, the gel need only be of the total RNA before poly A selection. Greater than 50% of EtBr stained material must be 28S and 18S bands.
- ☐ **f**. Screw-cap tubes (with O-ring) neatly labeled with sample name, date, and RNA concentration.
- ☐ **g**. Tubes shipped inside 50 mL conical tubes on dry ice.
- ☐ **h**. Completion of **ALL** tables in this form.

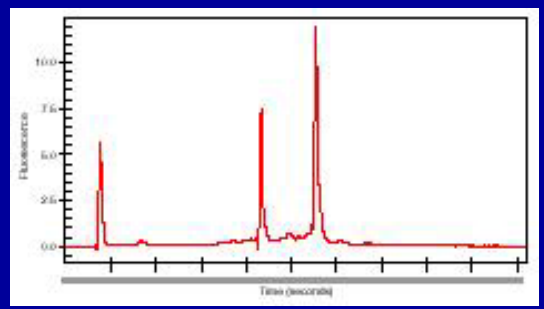
- New RNA amounts
- Clear Labeling
- Diluted Sample for Bioanalyzer
- Original Gel Image

RNA Isolation and Submission

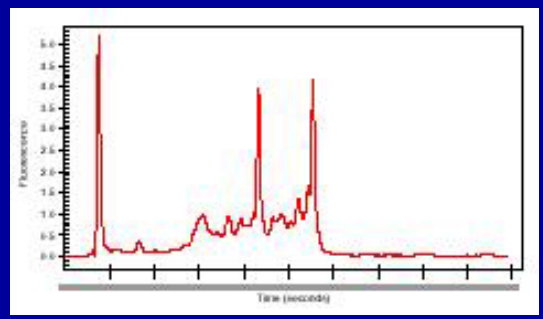
- Agilent 2100 Bioanalyzer
- Measure RNA quality and quantity
- Uses small sample size and take minutes



Agilent Gel Image



Good Quality RNA



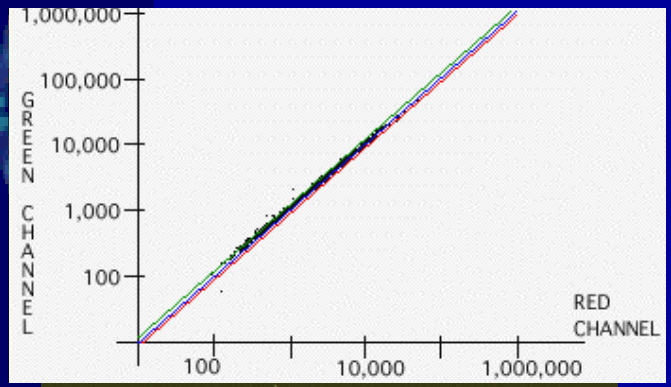
Degraded RNA

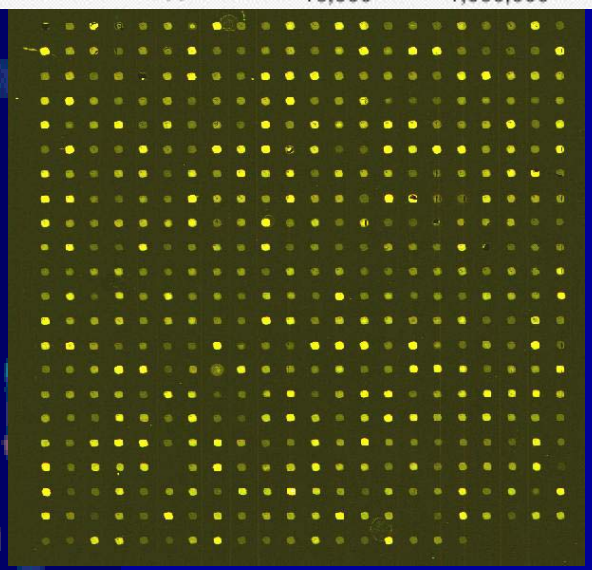
RNA Labeling Methods

- Direct Labeling
  - 25-50 ug per Channel
  - Depends on RNA Quality
- Indirect Labeling
  - Advertises 5-20 ug per Channel
- RNA Amplification
  - Advertises 1-10 ug per Channel
- Dendrimer Probes
  - Advertises 1-2 ug per Channel

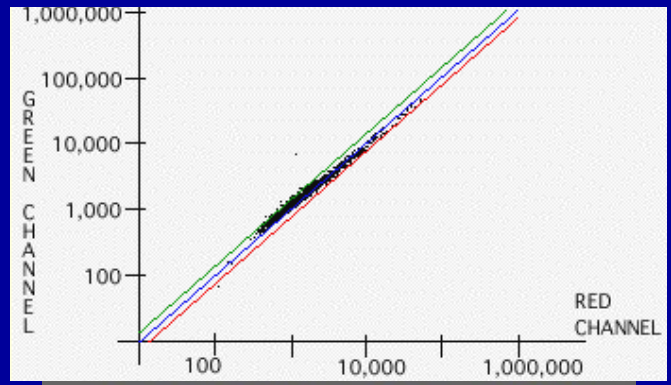
RNA Labeling Methods

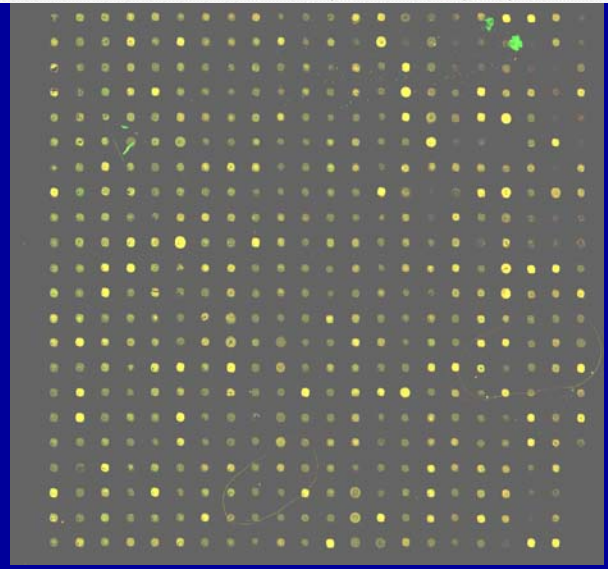
Direct Labeling 35 ug





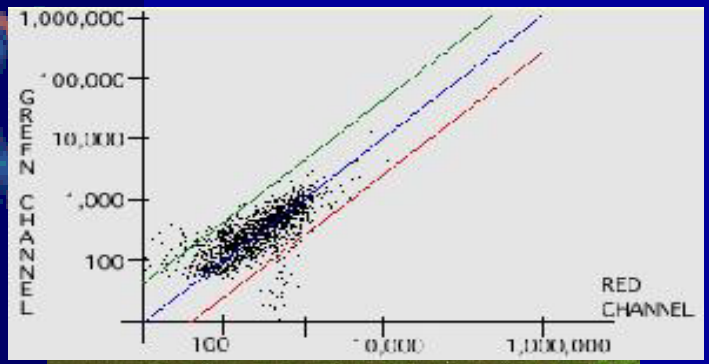
Indirect Labeling 20 ug

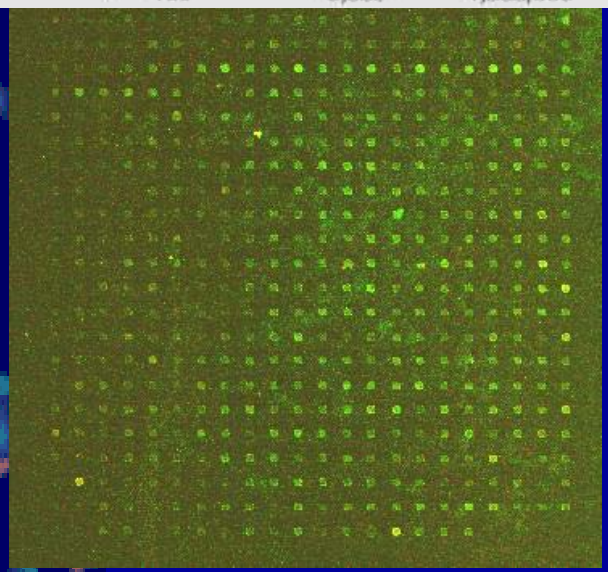




RNA Labeling Methods

Dendrimer Probes



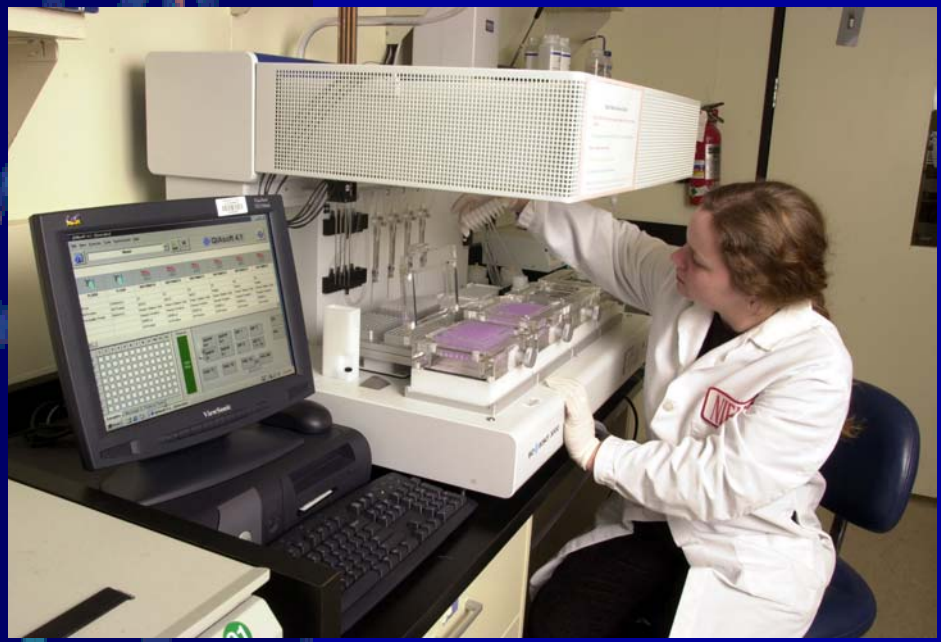


RNA Amplification

This is on the

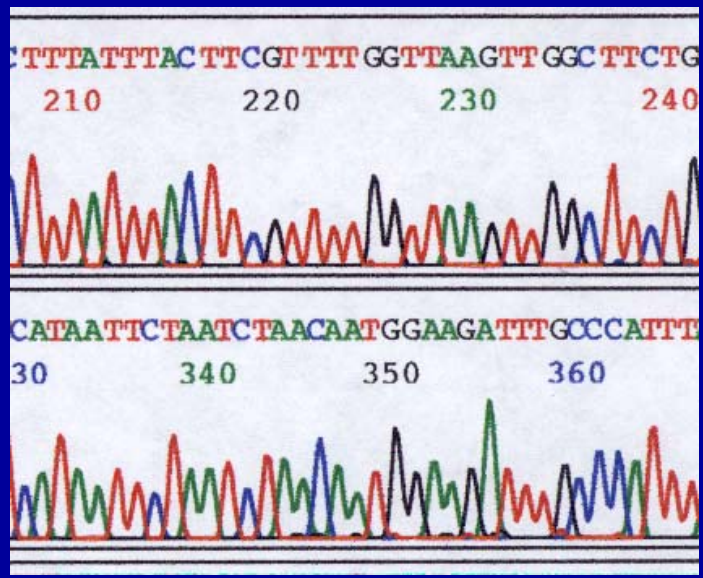
"To Do List"

High Throughput Sequencing



- Human Clones 85.22%
- Rat Clones 95.83%
- Mouse Clones 75.0%

High Throughput Sequencing



Using the Qiagen Biorobot we are currently sequencing about 400 clones per week.

Scanning and Image Analysis

NIH Scanner

2 Hours per Slide

Axon

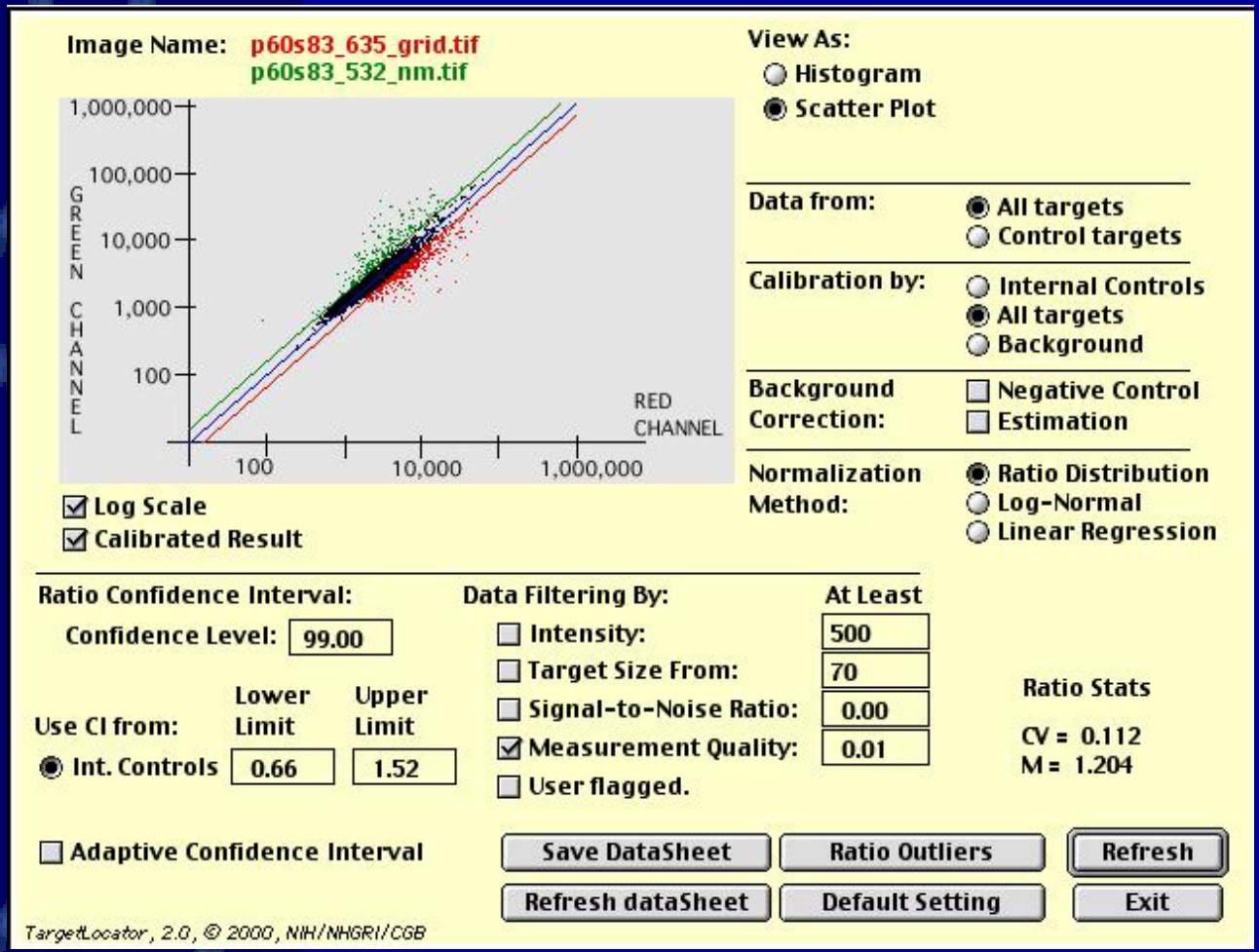
20 minutes per slide

Scanning and Image Analysis

- 5 um Resolution
- 7 minutes per slide
- 48 slide capacity
- Confocal Scanning with Autofocus
- Automated Image Analysis coming soon

Scanning and Image Analysis

ArraySuite 2.0

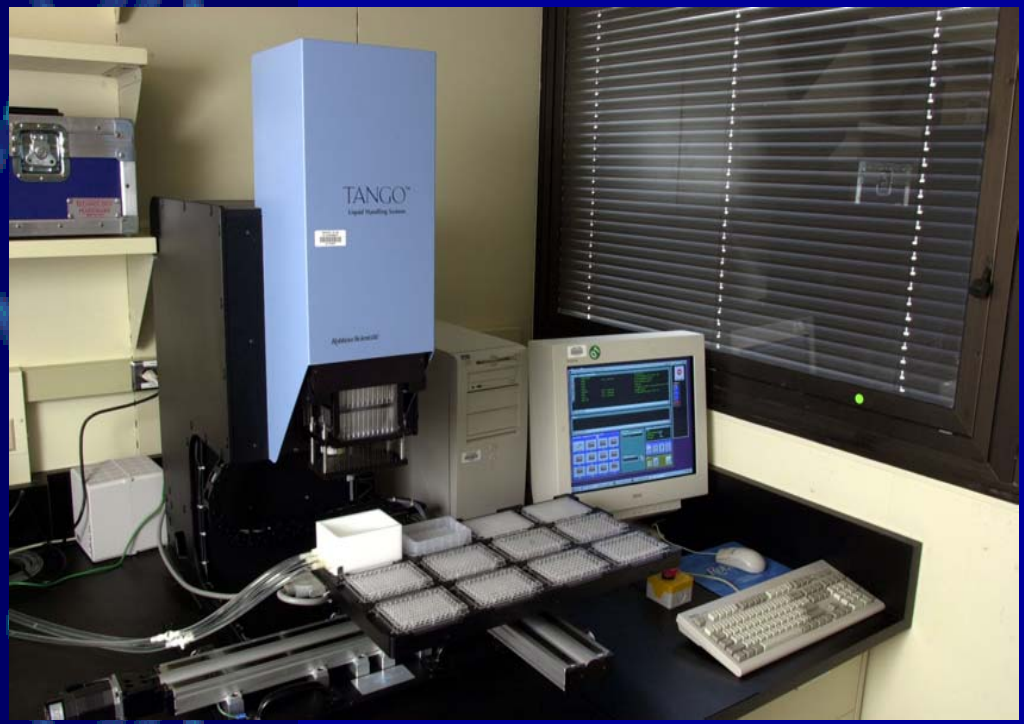


- Handles 32 pins
- Quality Metric
- Linear Regression
- Adaptive Confidence Limits
- Easier Gridding

Printing Modifications

- Beecher Instruments
- 96 Slides
- Tortoise and the Hare

Printing Modifications



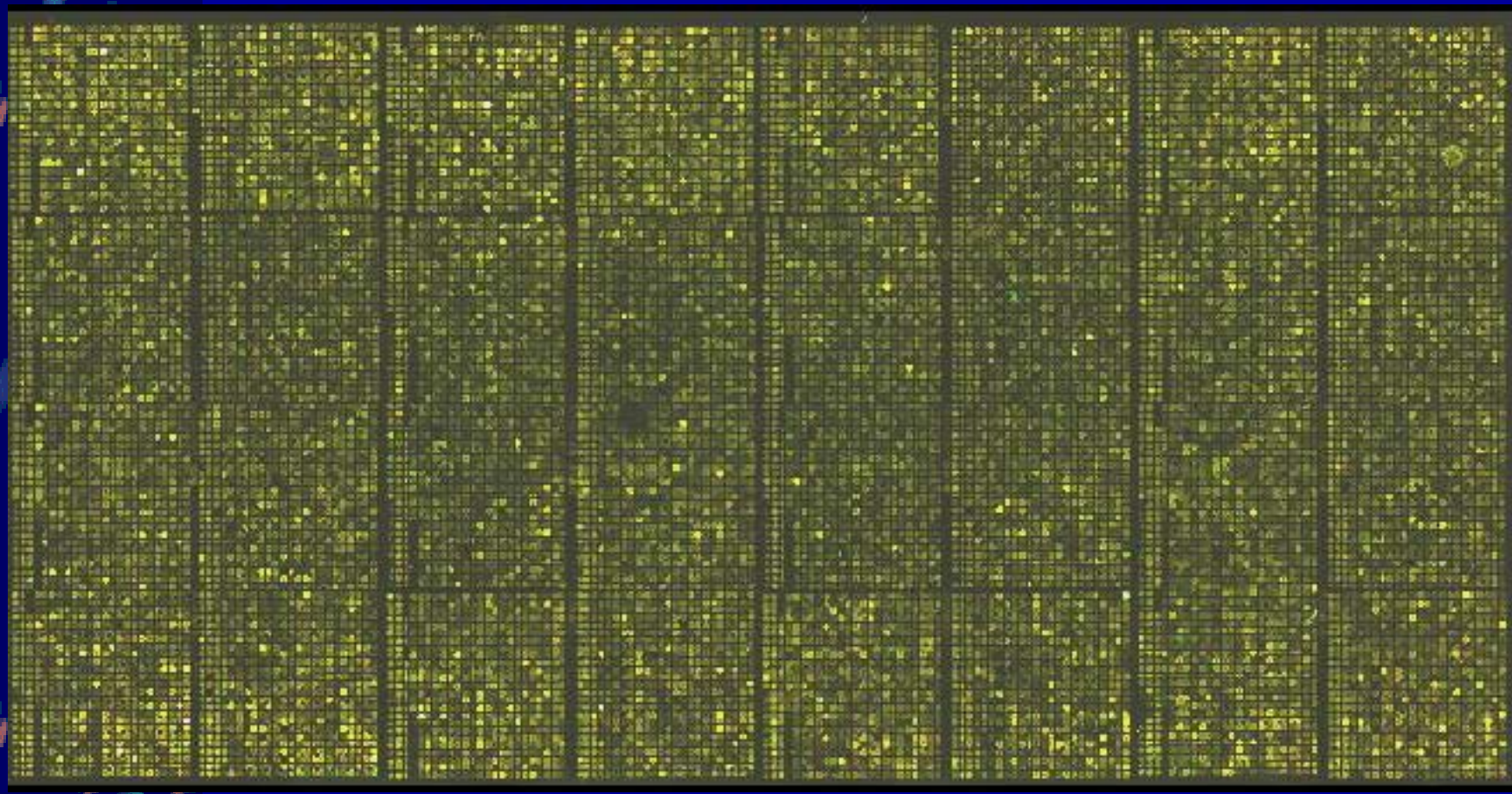
Making our Tortoise a Hare

- Accurate at small Volumes
- Used for Print Plate Refreshes
- Used for 96 to 384 plate conversions

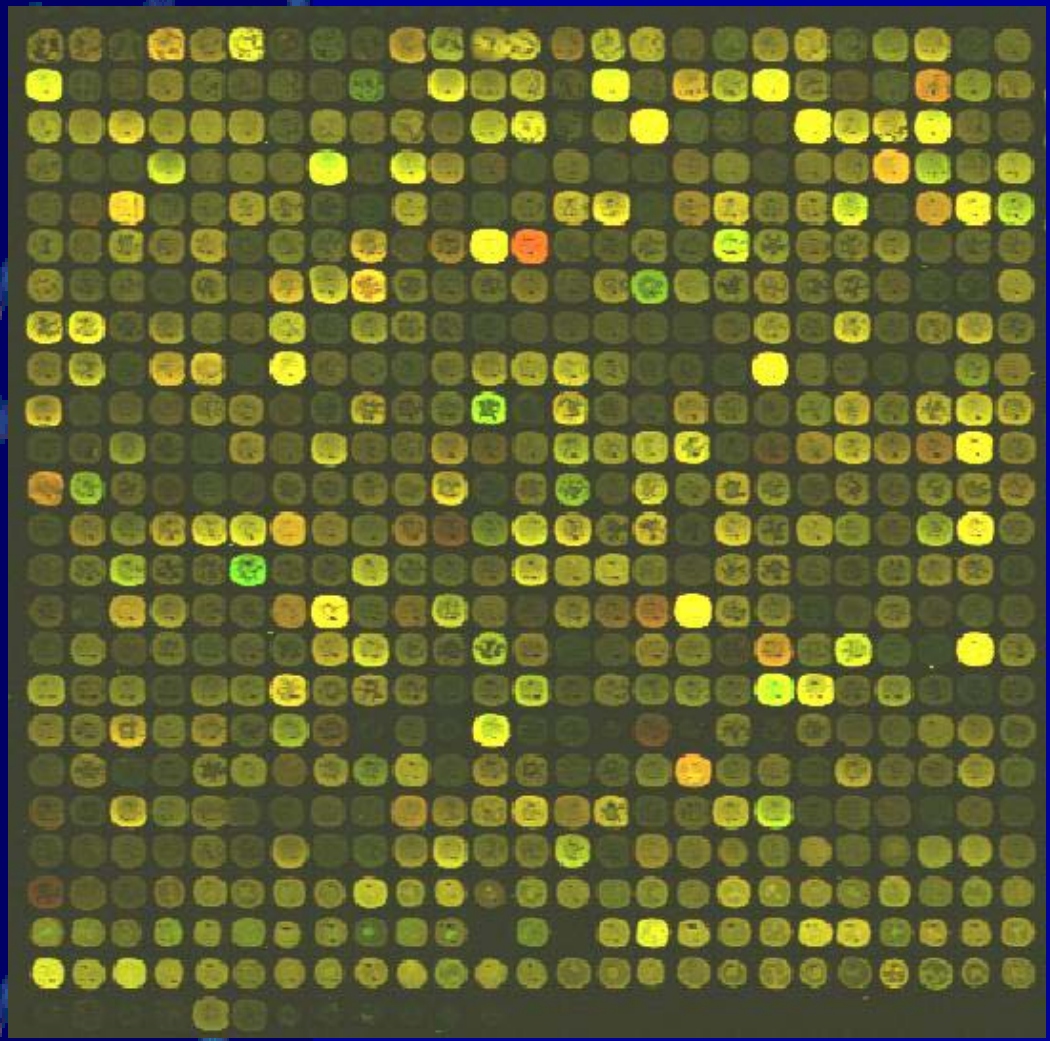
Printing Modifications

- 32 pin Print Head
- Allows use of 384 printing plates
- Basin Pin Wash
- New Vacuum Valve
- Can print 20,000 per slide spots in 1 week

The New Human Oligo Chip



The New Human Oligo Chip



- 17,000 70mer Oligos from Operon
- 2,400 cDNA from the NIEHS ToxChip
- 200 custom add ons from NCI and NIEHS
- The First to add cDNAs and Oligo to the same chip

Summary

- Reducing RNA amounts
- Constantly Testing New Protocols
- Adding New Technologies such as 70mer Oligos

Acknowledgements

Acknowledgements

Pete Cozart

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